

Growth and Development of the Lymphoid Organs as Affected by Inorganic and Organic Zinc in Broiler Chickens

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ABSTRACT

Zinc is one of the important minerals that affect the immune system. It should be incorporated in the diet since the effects of deficiency are very pronounced. However, the levels and sources are still unknown particularly in the growth and development of the lymphoid organs. Hence, this study was conducted. Two experiments were conducted following a completely randomized design for a period of 36 days. Experiment 1 utilized zinc oxide while 2 made used of zinc methionine. Results revealed that the different levels of zinc oxide did not affect the average weight and number of lymphatic nodules of the Bursa of Fabricius and thymus. The same organs involuted between 15-28 days and 11-21 days, respectively. The spleen, however, was unaffected. In Experiment 2, it shows that the average weight and number of lymphatic nodules of the bursa of Fabricius and thymus were not affected by the different levels of zinc methionine. Bursa involuted from days 8-28 while thymus, between 11-21 days. Increasing spleen's weight pattern was noted only in the control group beginning on day 11 and peak on day 28. The study concludes that the growth and development of the lymphoid organs were not affected by zinc inclusion in the diet. It recommends that supplementation must be accompanied by good management practices.

Keywords - Animal Nutrition, mineral supplementation, experimental design, Taipei, Taiwan, Republic of China

INTRODUCTION

In both human and animal's body, the immune system must effectively function to evade any agent that may cause diseases. However, this ability is dependent on the availability of the different systems. Some are broadly effective against a range of invaders while others are specific for individual infectious agents. Some act on the body surface while others act deep within the body to destroy organisms that succeed in breaching the outer defenses. The defenses of the body form a complex system of overlapping and interlinked mechanism that together will be able to control almost all invaders. If the defenses failed either because of the immune system destruction as seen in Acquired Immunodeficiency Syndrome (AIDS) or the invading organisms overcome or evade defenses as in cases of rabies, it will inevitably result to death. The immune system is not simply a useful system to be around, but it is essential to the life itself (Tizard, 2000).

Because of the many threats to human health for the last 20 years, including the increasing number of cancer cases, diabetes, hypertension, etc., people became more conscious to their health resulting to the advent and patronage of organic foods, including human and animal feed supplements.

One of the supplements they have given attention was zinc. In the developing countries, zinc deficiency is a major cause of childhood morbidity and mortality (Young *et al.*, 2014) and the deficiency is widespread throughout the developing countries affecting over two billion people (Prasad, 2008). Inadequate zinc dietary intake is influenced by other several intrinsic and extrinsic factors (Gibson, Hess, Hotz & Brown, 2008). The mineral is recognized as an essential trace element for all organisms and plays an important role in the development and integrity of the immune system affecting both innate and adaptive immune responses (Prasad 2000; Ibs, Gabriel & Rink, 2002; Bogden, 2004; Haase, Mocchegiani, & Rink 2006b; Mocchegiani, Cipriano Giacconi, & Malavolta, 2009). Both WHO/UNICEF and the 2008 Copenhagen Consensus Meeting recommend zinc supplementation in response to diarrhea, and to have a cost-effective strategy for advancing child welfare (Young *et al.*, 2014).

Zinc is one of the supplements in the human body and part of the ration in animal nutrition which its value has been recognized. However, the optimum dosages of the inorganic zinc oxide and newly introduced organic zinc methionine

in the market have not yet been established to ensure safety to the consumers. Moreover, since zinc is an integral part of protein synthesis which is involved in the folding of newly formed proteins and subsequently, antibody formation and activation of cellular immune responses. It was, therefore, necessary to determine the level of the mineral that would influence the growth and development of the primary and secondary lymphoid tissues which are the first lines of defense of the chickens against any antigen.

The study, therefore, aims to determine the effect of supplementing zinc in the form of zinc methionine or zinc oxide in the growth and development of lymphoid organs which are part of the body's immune system.

OBJECTIVE OF THE STUDY

The study aimed to determine the effects of the different levels of zinc oxide and zinc methionine on the growth and development of the broiler's lymphoid organs. Specifically, it aimed to determine the weight of the bursa of Fabricius, thymus and spleen, number of lymphatic nodules the time of bursal and thymus involution.

MATERIALS AND METHODS

Acquisition of Test Materials

Two zinc preparations were used in the study. Zinc oxide containing 81.4% zinc and zinc methionine containing 16% zinc with a purity of 99% and 100%, respectively. Other feed ingredients were purchased and stored in a refrigerated (4°C) stock room until they were used.

Experiment 1. Zinc oxide

A total of 230 day-old straight run Cobb broiler chicks were randomly distributed into four treatments following a Completely Randomized Design (CRD) in eight stainless cages with 16 birds per cage.

Each treatment was replicated twice, and the dietary treatments were as follows:

Treatment	Description
Treatment 1	Control diet, without additional zinc oxide
Treatment 2	Diet with 40 ppm zinc oxide (32.23 ppm zinc)
Treatment 3	Diet with 80 ppm zinc oxide (64.47 ppm zinc)
Treatment 4	Diet with 120 ppm zinc oxide (96.70ppm zinc)

Experimental Diets

The broiler booster, starter and finisher diets (Table 1) were formulated following the US-NRC's (1994) recommendation to contain 22.89%, 20.14% and 18.23% crude protein (CP) and 3000 metabolizable energy (ME kcal/kg), respectively.

The basal diet was composed mainly of ingredients of plant origin. All diets were fortified with amino acids, vitamins and minerals to meet the recommended nutrient requirements of the birds. Treatment 1 served as the control group and contained no additional exogenous source of zinc except the zinc of the basal diet. Treatments 2, 3 and 4, however, were supplemented with 40, 80 and 120 ppm zinc oxide or zinc methionine equivalent to 32.23, 64.47 and 96.70 ppm zinc in addition to the zinc content of the basal diet.

Feeding Trial

The day-old broiler chicks were brooded for three weeks. Chick booster was fed for 20 days and on the 21st to 28th day, starter feeds were offered. On the 22nd day, they were transferred to floor cages. On the 29th to 42nd day, finisher feeds were offered. Feeds and water were made available to the birds at all times.

During the first seven days, air temperature was maintained at around 35°C and then 30°C and finally, 25°C on day 23 onwards. As part of the management practice, Newcastle Disease (NCD) vaccine was administered to all birds intraocular on days 6 and 20.

On days 4, 8, 11, 15, 21 and 28, 3 birds from each treatment were randomly selected and sacrificed to determine the growth of the lymphoid organs. During each collection period, representative birds were weighed to compute for the relative growth percentage of the lymphoid organs. Each bird was carefully dissected to obtain the bursa of Fabricius, spleen and thymus. The organs were weighed using a digital weighing scale and preserved in a clean properly labeled bottle with 10% buffered formalin for histopathological processing.

Table 1. Ingredient composition (%) and calculated nutrient content of the basal diet

INGREDIENTS	BOOSTER	STARTER	FINISHER
Corn	61.1	64.5	54.4
Soybean meal	28.0	27.5	28.3
Soybean protein	5.0	2.8	
Wheat bran			6.9
Limestone	1.5	1.4	1.3
Calcium phosphate	1.4	1.3	1.0
Salt	0.2	0.2	0.2
Vitamin premix ¹	0.1	0.1	0.1
Mineral premix ²	0.1	0.1	0.1
Methionine	0.2	0.1	0.1
Choline chloride	0.1	0.1	0.1
Soybean oil	2.2	1.8	7.4
Calculated Nutrient Content	100	100	100
Crude protein (%)	22.89	20.14	18.23
Metabolizable Energy (Kcal/kg)	3005.00	3004.00	3003.00
Calcium (%)	0.97	0.91	0.83
Non-phytate phosphorus (%)	0.15	0.13	0.11
Phytate phosphorus (%)	0.35	0.37	0.40
Lysine (%)	1.20	1.00	0.94
Threonine (%)	0.82	0.75	0.67
Tryptophan (%)	0.28	0.26	0.24
Arginine (%)	1.44	1.30	1.12
Isoleucine (%)	0.87	0.81	0.74
Valine (%)	1.40	0.95	0.85
Sodium (%)	0.11	0.11	0.11
Chloride (%)	0.23	0.23	0.19
Crude fiber (%)	3.31	3.30	3.93
Zn (ppm) ³	35.16	36.36	38.32

¹ Vitamin premix provided the following per kg of diet: Vitamin A: 5000 IU; Vitamin D 500 ICU; Vitamin E: 20 IU; Vitamin K1: 1 mg; Thiamin: 1.8 mg; Riboflavin 5.4 mg; Niacin: 27 mg; Vitamin B: 4.2 mg; Vitamin B12: 0.03 mg; Folic acid: 0.55 mg; Biotin: 0.16 mg; Ca pantothenate: 10 mg

²Trace mineral premix supplied the following per kg of diet: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 34.9 mg, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 207.2 mg, Na_2SeO_3 0.4 mg, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 442.5 mg.

³The computed zinc contents of the basal diets obtained from the different zinc contents of the ingredients. On top of it zinc methionine (16% zinc) was added at 40 ppm, 80 ppm and 120 ppm in the different treatments to 6.4 ppm, 12.8 ppm and 19.2 ppm for Treatments 2, 3 and 4

Parameters Gathered

Weight of the organs. In each collection period, freshly dissected organs were weighed using a digital weighing scale. As for the thymus, it was expressed as the average weight of the left and right thymi.

Number of lymphatic nodules. The number of lymphatic nodules presented as the average number of the lymphatic nodules counted from five microscopic fields of the prepared slides. Reading was made using the low power objective of a compound microscope.

The time of bursal and thymus involution. The involution of the bursa and thymus was determined by comparing the weights of the organs from days 4 to 28 as well as the number of their respective lymphatic nodules. The lightest weight and least number of lymphatic nodules after a successive increase or decrease in weight of the organ or number were considered as the time of involution.

Chemical Analyses

The plasma zinc content was analyzed using Atomic Absorption Spectrometer (Perkin Elmer Analyst 200°, Perkin Elmer Corporation, USA) following the protocol of AOAC International (2012).

Ethical Considerations

The trial protocol in the study was approved by the Institutional Animal Care and Use Committee (IACCUC) of the National Taiwan University, Taipei, Taiwan, Republic of China.

Data Analysis

All data gathered in this study were analyzed using one-way analysis of

variance following the Complete Randomized Design (CRD). Comparison of treatment means were subjected to Duncan's Multiple Range Test (DMRT). Statistical significance was established at 0.05 level.

Experiment 2. Zinc Methionine

Experimental Design and Birds

The experimental design, number of birds used and replications were similar to that in Experiment 1 except for the source of zinc. The four (4) treatments are as follows:

Treatment	Description
Treatment 1	Control diet, without additional zinc
Treatment 2	Diet with 40 ppm zinc methionine (6.4 ppm zinc)
Treatment 3	Diet with 80 ppm zinc methionine (12.8 ppm zinc)
Treatment 4	Diet with 120 ppm zinc methionine (19.2 ppm zinc)

Treatment 1 in this experiment utilized the same control group used in Experiment 1.

Experimental Diet

Ingredient composition and nutrient content of diets were the same as that in Experiment 1 except that zinc methionine was used instead of zinc oxide. Since the zinc content of zinc methionine was 16%, and the purity was 100% with the inclusion of 40 mg, 80 mg, and 120 mg of zinc methionine, the additional zinc content of the diets were 6.4 ppm for Treatment 2, 12.8 ppm for Treatment 3 and 19.2 ppm for Treatment 4.

Feeding Trial

Same procedural management was followed as in Experiment 1.

Parameters Gathered

All data gathered in this experiment were similar to that in Experiment 1

RESULTS AND DISCUSSION

Experiment 1. Zinc oxide

Growth and development of the bursa of Fabricius

Figures 1 and 2 show the average weight and number of lymphatic nodules in the bursa of Fabricius of broilers fed diets with zinc oxide at different ages from day 4 to day 28. Although it appears that numerically, there is difference among the treatments, the presence of high standard deviation on statistical analysis resulted in the absence of significant difference on the weights of the bursa and other parameters among treatments. Generally, the increasing weight was noted from day 8 onwards in all treatments. This indicates that the different levels of zinc oxide have no direct effect on the weight of the bursa of Fabricius. Pimentel, Cook, and Greger (1991) reported that chicks fed diets with 8 μg zinc/g had smaller bursae as compared with those fed 18 μg zinc/g or higher for five weeks but at seven weeks, the chicks fed 8 μg zinc/g had larger bursae than the other chicks. The present study shows an increasing growth pattern of the bursa of Fabricius from ages 4-28 as reflected in Figure 1 in all treatments. On day 21, Treatment 3 manifested the heaviest bursa that can be due to strong antigenic stimulation. What made it heavy on day 28 was apparently due to the development of its capsule (see Figure 4).

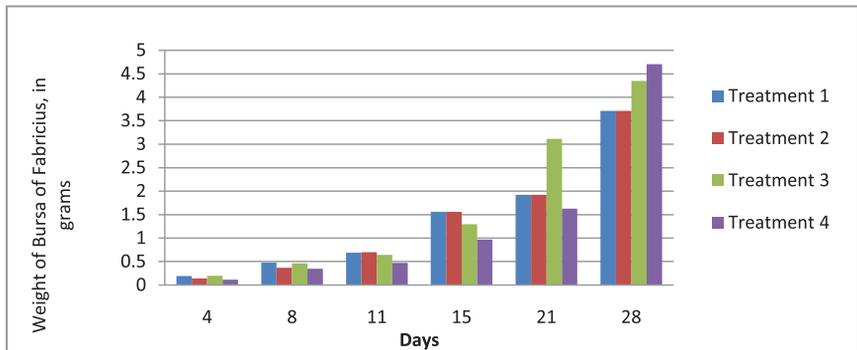


Figure 1. Average weight (grams) of the bursa of Fabricius of broilers fed zinc oxide.

Shown in Figure 2 are the average numbers of lymphatic nodules in the bursa of Fabricius at different ages. It appears that there was a declining number beginning on day 8 up to day 21 for Treatments 3 and 4 whereas for Treatment 1, it was on day 11 and day 15 for Treatment 2. The declining number of lymphatic nodules from days 4 to 28 can be due to apoptosis or physiological cell death or suicide mediated by normal processes (Tizard, 2000) as observed by the degeneration of the lymphocytes it contains (see Figure 4). The findings conform to the findings of Pimentel, Cook, and Greger (1991) that low zinc intake may delay the normal atrophy of the lymphoid organs as the animal matures.

Delaying the normal atrophy of the lymphoid organ is very important because it shows that somehow, it would still be capable to protect the animals in their crucial stage of life which in broiler, it is about 15-21 days old when they are most susceptible to infection. The bursa of Fabricius is a primary lymphoid organ that functions as a maturation and differentiation site for the cells of the antibody-forming system particularly known as B lymphocytes. In addition, the bursa can also trap antigens and undertake some antibodies synthesis and can elicit the proliferation of many lymphocytes (Tizard, 2000) that will add up to the weight of the organ. Based on the lowest number of lymphatic nodules followed by an abrupt increase in growth or number of the same the following day, it appeared that the bursa involuted on days 28, 15, 15 and 21 for Treatments 1, 2, 3 and 4, respectively.

The study conforms to the many claims of both broiler raisers and veterinary practitioners that susceptibility of broilers to infection begins on day 15 to day 21 because during this period, the bursa which is supposed to protect the animal from disease, begins to involute.

The non-significant result in the number of lymphatic nodule in the present study can be implicated by being apparently healthy of the birds throughout the duration of the study in which there was a continued growth until day 28.

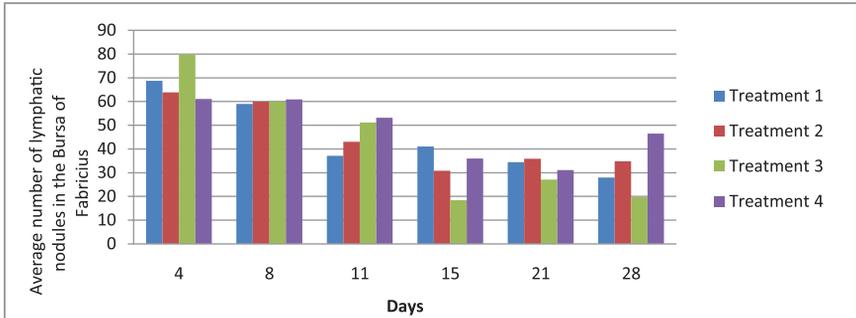


Figure 2. Average number of lymphatic nodules in the bursa of Fabricius of broilers fed zinc oxide



Figure 3. Photomicrograph of the cross-section of the bursa of Fabricius of a four day-old broiler fed zinc oxide containing: a) well-developed lymphatic nodules; and b) germinal centers at 100x magnification

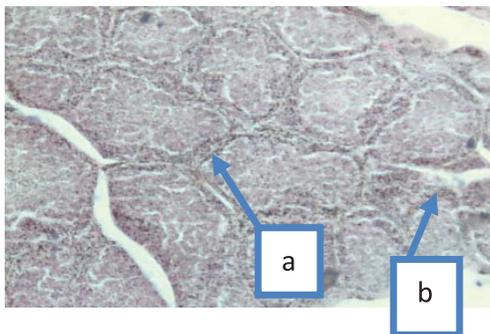


Figure 4. Photomicrographs of the cross section of the bursa of Fabricius of broilers fed zinc oxide on day 15 with signs of degeneration such as: a) thickening of the nodular capsules and b) disappearance of the nodule's contents at 100x magnification

Growth and development of the thymus

Shown in Figure 5 are the average weights and numbers of lymphatic nodules in the thymus of broilers fed diets with zinc oxide at different ages. As shown, the weights of thymus for the different treatments are almost the same with each other on day 4. Increasing growth was noticed at the beginning of day 8 onwards. Statistical analysis revealed that there was no significant difference ($P>0.05$) among treatments from four to 28 days of age.

The study showed that the different levels of zinc oxide in the ration did not affect the weight of the thymus from day 4 to day 28. The increasing pattern in the weight of the thymus conforms to the statements of Eroschenko and Di Fiore (2013) and Tizard (2000) that the gland attains its greatest development shortly after birth until puberty and then involutes thereafter. In this study, the growth of thymus peaks on day 28. The growth observed during this period can be considered as part of normal physiological mechanism where there is an enlargement of the gland's capsule that added weight to the organ.

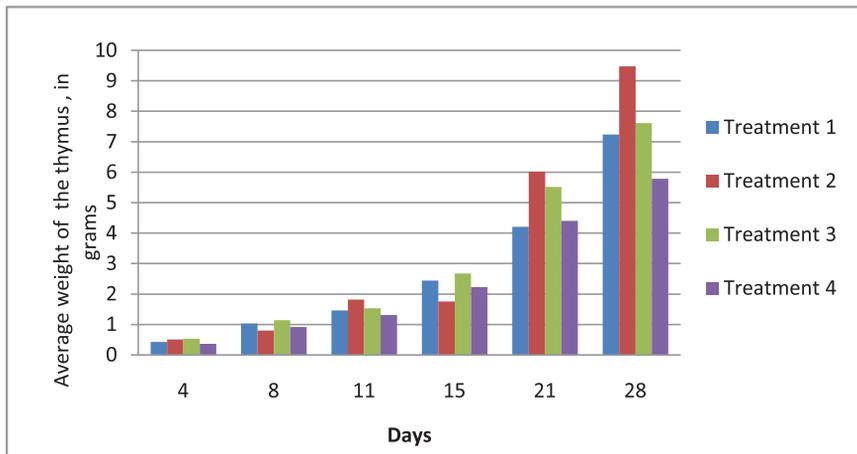


Figure 5. Average weights (grams) of the thymus of broilers fed zinc oxide

However, since it is a site of lymphocytes' development and maturation, there is also a possibility that the animals encountered antigen that allowed the surge of lymphocytes in the organ thus, adding weight into it. Lymphocytes are responsible for the acquired immune responses; small round cells that predominate in organs such as the spleen, lymph nodes, and thymus. They have antigen receptors on

their surface and can therefore, respond to antigens thus, are called antigen-sensitive cells. They are also responsible for the production of antibodies and for cell-mediated immune responses (Tizard, 2000). During the time of bursal involution, B-lymphocytes become less active while the T-lymphocytes in the thymus are being activated.

It appeared that on day 15, the weight of the thymus is just beginning to gain. This period is the time where birds are susceptible to whatever form of infection owing to the decline in the innate immunity. During the first week of life, the immunity of the birds is still high due to the protection derived from the hen through the yolk. Within the egg, the antibodies transfer to the albumin by four days and to the embryo by 12 days. This passive immunity makes the newly hatched chick resistant to successful vaccination in the same manner as young mammals (Tizard, 1987). On day 21, as appeared in Figure 6, there was a gradual increase in the weight of the gland probably a defense mechanism of the animal against infection. Tizard (1987) claimed that in chicks, immunity steadily declines after hatching and disappears between 10-20 days later. In the present study, it was observed between days 15 and 21. On day 21, the animal attempted to combat the infection. Since vaccination is a form of stressor to the birds and one of the booster shots was given on day 7, it is possible that the birds are immunosuppressed shortly days after that vaccination since immunity will be mounted at the next 7-10 days only. The decline in the growth percentage of the thymus can also be considered here; the immunity has not yet been mounted, but after which it was established apart from the normal physiological response.

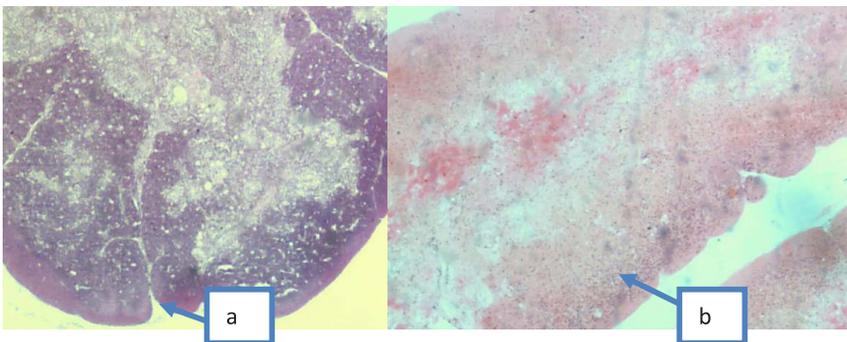


Figure 6. Photomicrographs of the cross-sections (a,b) of well-developed lymphatic nodules in thymi of eight-day old broilers fed zinc oxide at 100 magnification.

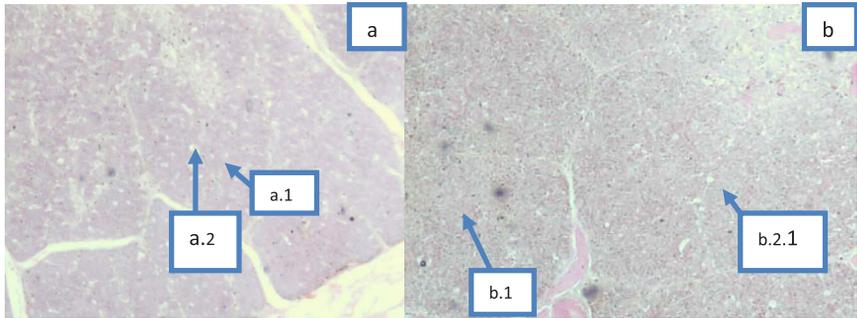


Figure 7. Photomicrographs of the cross-section of thymus of 21(a) and 28 (b) -day old broilers fed with zinc oxide with very (a.1 and b.1) dense lymphatic contents and prominent germinal centres (a.2 and b.2) at 100 magnification.

Average Number of Lymphatic Nodules in the Thymus

Shown in Figure 8 is the average number of lymphatic nodules in the thymus of broilers fed diets with zinc oxide at different ages. It shows that there was no significant difference was noted among the treatments. It also shows that there is no pattern observed in the number of lymphatic nodules. The declined in the number of lymphatic nodules on days 11, 15 to 21 can be correlated to the immunosuppressed state of the animal because of the lag period. The lag period is the interval between administration of antigen and the first detection of antibody. It is probable that the lymphocytes that have matured and developed in the organ already migrated towards an antigen, hence the decline in the number of lymphatic nodules. Lymphatic nodules contain germinal centers that in turn are the sites of developing lymphocytes. Although the puberty stage in broiler breeder is at 18 weeks where the thymus attains its peak size and eventually involutes, it shows from this study that the involution of the thymus starts as early as day 11 to day 21 as evidenced by declining number of lymphatic nodules. When the bird gets old, the thymus shrinks and gradually replaced by fat (Tizard, 2000).

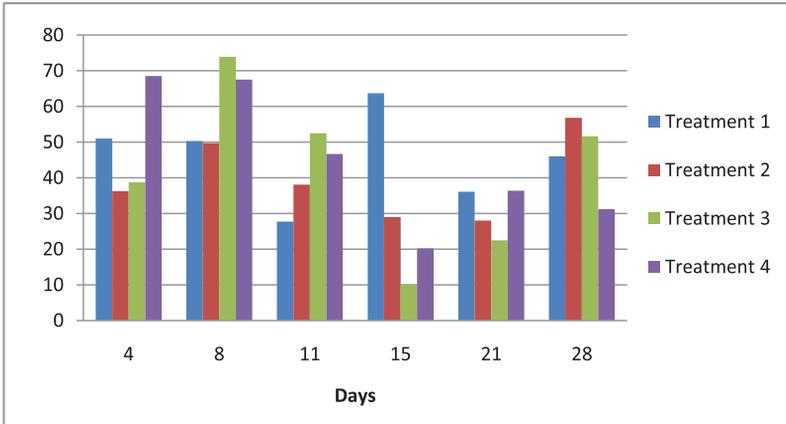


Figure 8. Average number of lymphatic nodules in the thymus of broilers fed zinc oxide

Average weight of the spleen

The average weight of the spleen of broilers fed zinc oxide is shown in Figure 9. Although there was no statistical difference noted on the weight of spleen among the treatments, an increasing pattern was noted on the weight of the organ. The slightly heavier weight of Treatment 1 in all the collection periods except on day 11 can be due to immunostimulation resulting to the surged of many lymphocytes, dendritic cells and macrophages that functions to remove foreign material from the blood (Tizard, 2000). The same observation was noted on day 15 where all of the treatments showed remarkable increased in weight as compared to day 11 and continued to increase in growth on days 21 and 28. Note that the number of lymphatic nodules both in the bursa of Fabricius and thymus declined on day 15 except for Treatment 1 which suggests that possibly, all their matured lymphocytes moved to the spleen in the mentioned period. Tizard (2000) stated that secondary lymphoid organs like the spleen enlarge in response to antigenic stimulation.

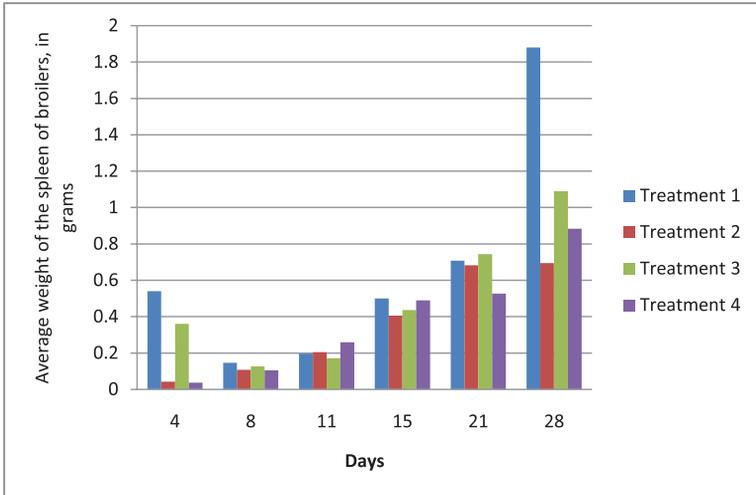


Figure 9. Average weight (grams) of the spleen of broilers fed zinc oxide.

Experiment 2. Zinc Methionine

Growth and Development of the Bursa of Fabricius

Figure 10 shows the increasing weight pattern of the bursa of Fabricius from days 4 to 28. This is contrary to the claim of Tizard (2000) that the birds attain their greatest size in 1-2 weeks after hatching and then shrinks/involutes as the birds' ages. In this study, the bursa of Fabricius kept on growing until day 28. Figures 11, 12a and 12b show the sudden changes in the features of the bursa of Fabricius from day 4 to day 21. On day 4, the lymphatic nodules are still prominent and the contents are still numerous, however, on day 21, the signs of involution already appeared such as thickening of the lymphatic capsules and disappearance of lymphatic contents.

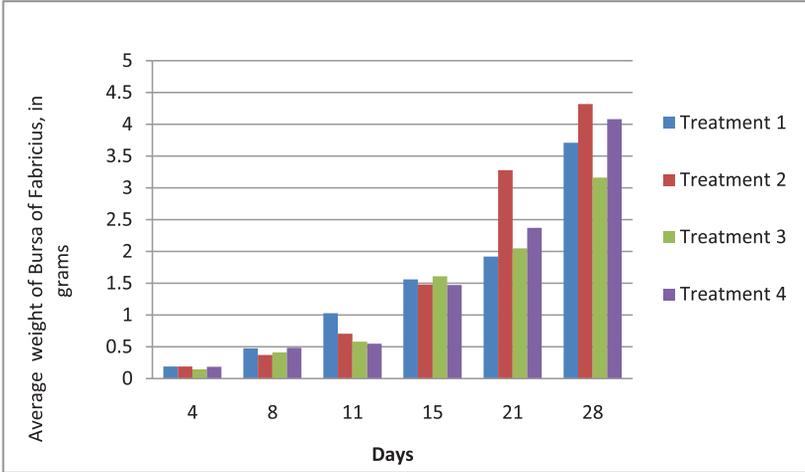


Figure 10. Average weigh (grams) of the bursa of Fabricius of broilers fed zinc methionine

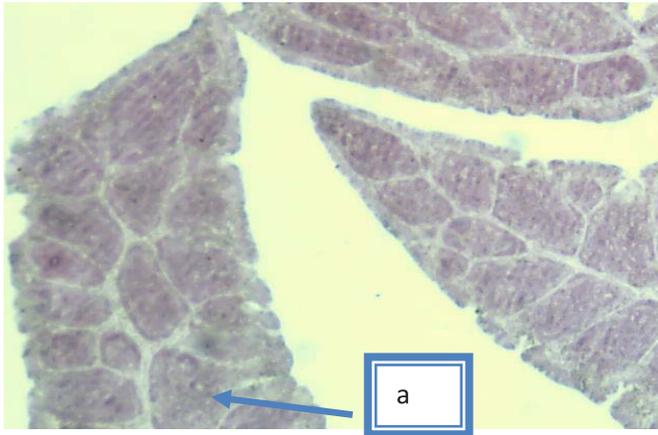


Figure 11. Photomicrograph of the cross-section of the bursa of Fabricius of broilers fed zinc methionine on day 4 showing the very (a) prominent lymphatic nodules at 100 magnification

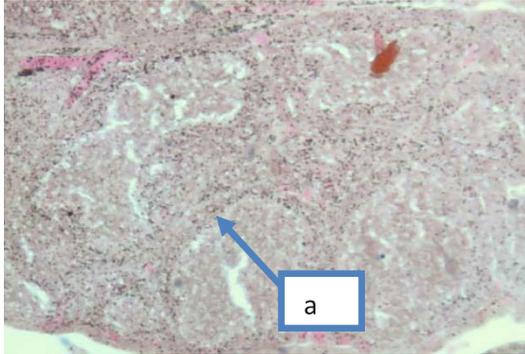


Figure 12a. Photomicrograph of the crossed-section of the bursa of Fabricius of broilers fed zinc methionine on day 21 showing signs of degeneration like (a) thickening of the capsule at 100 magnification.

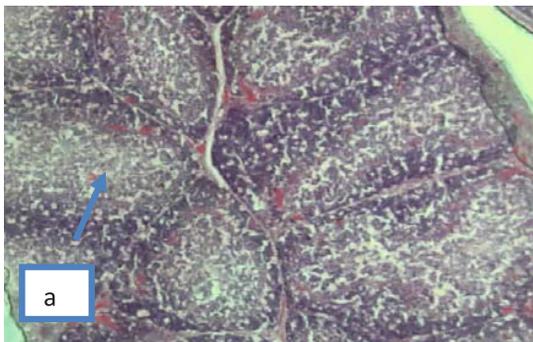


Figure 12b. Another photomicrograph of the bursa of Fabricius of broilers fed zinc methionine showing signs of degeneration like (a) disappearance of lymphatic contents.

Although days 15 and 21 are the critical periods of bursal growth owing to the susceptibility of the animal to infection, statistical analysis revealed that the different treatments did not vary. Since there were no discharges found in the organ, the growth can just be a normal physiological mechanism. This indicates a delay in the involution of the bursa of Fabricius. The implication of delayed involution would mean that there will be more antibody-forming cells that will be produced because the bursa is a primary lymphoid organ that functions as a maturation and differentiation site of the said cells. Immature cells from the bone

marrow migrate to the bursa and once matured, they migrate to the secondary lymphoid organs like the spleen, tonsils, lymph nodes etc. where they respond to antigens. The number of circulating lymphocytes will possibly decrease if involution occurs early. The capability to trap antigens and undertake some antibodies synthesis show that the bursa is not just a primary lymphoid organ (Tizard, 2000) which can possibly decrease the levels of antibodies at the early stage of life if they have involuted early. Pimentel, Cook, and Greger (1991) in their study found out that chicks fed low levels of zinc ($8\mu\text{g/g}$) had an overall delay in their growth and in the normal atrophy of the lymphoid organs as the animals mature. The present study also observed the same result even if the exogenous level of zinc methionine in the ratio is 19.2 ppm.

The sudden increase in growth until day 28 is possibly due to the encounter with antigens that allowed it to act as secondary lymphoid organ by trapping antigens and was able to undertake antibody synthesis that added weight to the organ. The delay in the involution of the bursa can be favorable because it would provide more sites for the lymphocytes to mature, and it would allow the organ to function as secondary lymphoid organ by trapping antigens and undertake some antibodies synthesis (Tizard, 2000).

Average Number of Lymphatic Nodules in the Bursa of Fabricius

Figure 13 shows that the different levels of zinc methionine did not influence the number of lymphatic nodules in the Bursa of Fabricius. During the first eight days of life, the numerous lymphatic nodules could be attributed to the growing bursa. These nodules are the sites of many germinal centers where immature lymphocytes derived from bone marrow migrate and eventually mature. However, the decreasing number of the lymphatic nodules beginning on day 15 can be associated to the degeneration of the bursa of Fabricius. Although it shows from Figure 10 that the bursa becomes heavier until day 28, it is probable that the capsule grew, but the lymphocytes started to migrate to secondary lymphoid nodules owing to exposure to an antigen or the organ itself started to involute beginning on day 15. The decreasing numbers of the lymphatic nodules on day 15 can be associated to the observation of many farm veterinarians and producers that days 15-21 are the most critical stages in the life of broilers. In the study, the signs of involution occurred four days later.

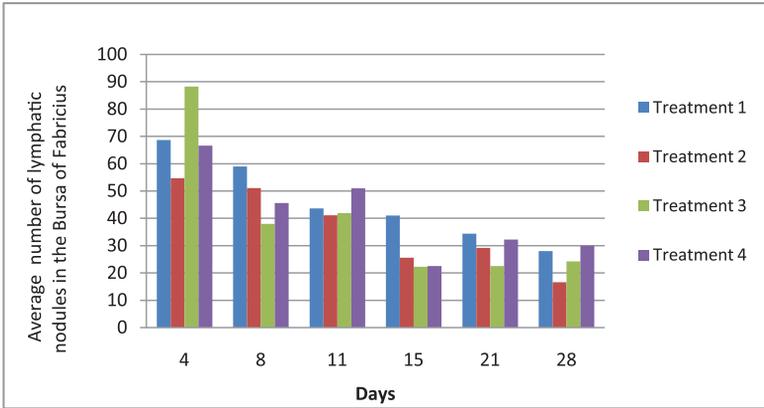


Figure 13. Average number of lymphatic nodules in the bursa of Fabricius of broilers fed zinc methionine

The day in which the lymphatic nodules declined and had the lowest number followed by the sudden increase in number is considered as the period of bursal involution. Figure 13 shows that the bursa involuted in a wide range of time from day 8 to even after day 28. Based on the number of lymphatic nodules, involution starts on day 8. This is very important because on days 15-21 broilers are predisposed to different kinds of antigens. The possible involution that occurred on day 8 in Treatment 3 can be attributed to the exposure of the birds to farm challenge especially it was winter during the time this experiment was conducted. Signs of delayed involution manifested by broilers in Treatment 1 or control group would mean a better immune system because the birds were still able to maximize the time to develop and mature lymphocytes in the bursa of Fabricius.

Growth and Development of the Thymus

Figure 14 shows the average weight and number of lymphatic nodules of the thymus of birds fed diets with zinc methionine at different ages. Like the previous experiment, there was no significant difference on the average weight of the thymus at different collection periods although there was an increasing pattern noted except on day 28.

The findings show that birds fed different levels of zinc methionine in the ration did not influence the weight of the thymus of broilers. It further shows that the thymus increases in weight as the bird matures from day 4 until day 21.

The increasing weight of the thymus from day 4 until day 21 (Figure 14) can be associated to the possible immunostimulation of the birds resulting to the migration of mature lymphocytes from the Bursa down to the thymus. The migration of the lymphocytes and other dendritic cells eventually led to an increasing weight of the thymus until day 21. On day 28, the dropped in weight can be associated to the normal physiological mechanism.

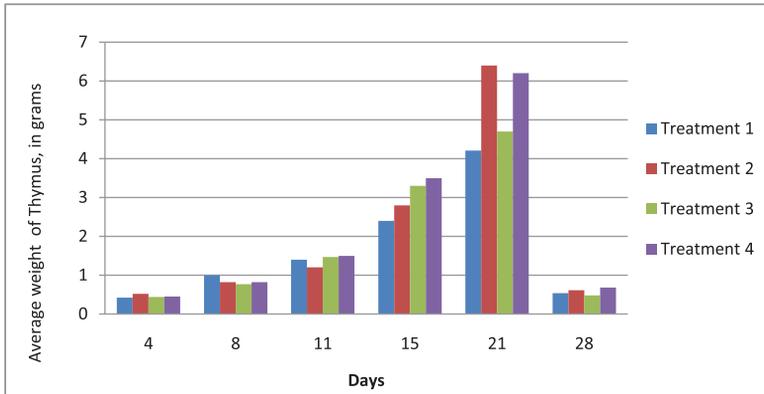


Figure 14. Average weight (grams) of the thymus of broilers fed diets with different levels of zinc methionine

Tizard (2000) and Eroschenko and Di Fiore (2013) stated that the involution of the thymus occurs at the stage of puberty. However, day 28 is too early to be claimed as the puberty stage of broiler breeder which is at week 18.

Average Number of Lymphatic Nodules in the Thymus

Figure 15 shows the average number of lymphatic nodules in the thymus of broilers fed diets with zinc methionine from days 4-28. No statistical significance was noted among the treatments which indicates that the inclusion rates used did not affect the number of lymphatic nodules in the thymus as evident by the lack of pattern.

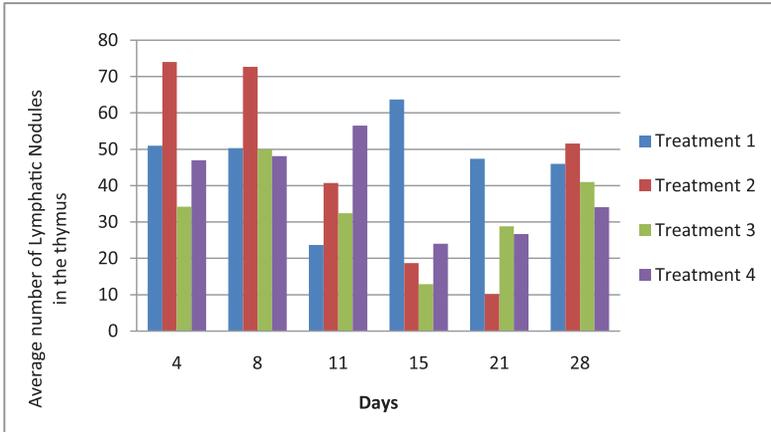


Figure 15. Average number of lymphatic nodules in the thymus of broilers fed diets with different levels of zinc methionine

The least numbers of lymphatic nodules were between days 11 to 21. During the first few days of life, the lymphocytes are still developing and maturing in the primary lymphoid organs like the bursa of Fabricius. Between days 15-21, presumably, the lymphocytes are in the process of maturation and fully mature on days 21 and 28 which eventually fill-in the lymphatic nodules.

The study reveals that broilers fed diets with zinc methionine regardless of level involuted on days 15 and 21 compared to day 11 of Treatment 1/Control or those that did not receive an exogenous source of zinc. This is contrary to the observation of Pimentel, Cook, and Greger (1991) that chicks fed low level of zinc would have a delay in the involution of both thymus and bursa of Fabricius.

Average Weight of the Spleen

Figure 16 presents the average weight of the spleen of broilers fed diets with different levels of zinc methionine. Results showed that broilers in all treatments did not differ statistically on the average weight of the spleen. The organ continued to grow from days 4-28 in all treatments with statistical difference noticed on day 11 only between treatments. Treatments 2-4 were not different from each other, but they are all different to Treatment 1.

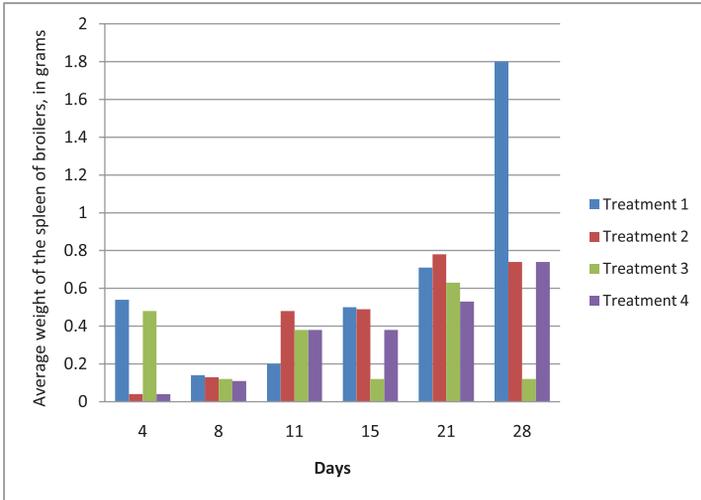


Figure 16. Average weight (grams) of the spleen of broilers fed diets with different levels of zinc methionine

The sequence of events in this study conforms to the findings of other researchers that the bursa of Fabricius and thymus act as primary lymphoid organs and the spleen as the secondary lymphoid organ (Tizard 2000; Eroschenko & Di Fiore, 2013). Based on the result, the primary lymphoid organs are very active during the first two weeks of life and then, suddenly involuted which on the other hand, signals the growth and development of the secondary lymphoid organs like the spleen by the migration of lymphocytes and other antigen presenting cells. The sudden growth of the spleen depicted in Figure 16 on day 11 is a manifestation that the spleen functions as a secondary lymphoid organ. On day 15, there was a sudden increase in the weight of the spleen of broilers in the control group and to the group that have received 40 ppm of zinc methionine which may be due to strong antigenic stimulation. The increase in the 21st and 28th day in the spleen's growth can be associated with stronger antigenic stimulation. One of the functions of the spleen is to filter blood and as such, it is also capable of trapping antigens. Trapping of antigens in turn will possibly increase the number of lymphocytes that are capable of performing cell-mediated immune response.

CONCLUSIONS

Within the conditions under which these experiments were conducted, it is concluded that the different levels and sources of zinc do not affect the growth and development of the bursa, thymus, and spleen. However, bursal and thymus involutions on birds fed with zinc oxide occurred between 15-28 and 11-21 days, respectively. As for the zinc methionine fed broilers, the growth and development of the bursa of Fabricius, thymus and spleen manifested the same result in broilers fed with zinc oxide. Both bursa and thymus involution occurred between days 8 to-28.

TRANSLATIONAL RESEARCH

The findings of the present research can be used by animal nutritionists and farm veterinarians in designing a more holistic approach in safeguarding the health and welfare of the animals. Whatever the claimed benefits of any supplement is, whether it is in the form of a mineral or vitamins either in inorganic or organic forms, its full benefits can only be maximized if other factors like proper management will be observed. A similar research on a controlled environment is also encouraged to fully demonstrate the effect of the utilized mineral forms.

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